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The goal of this research is to expand the knowledge of the genes that contribute to neurofibromatosis beyond the GAP1-related domain in NF1. It is hypothesized that the gene encoding RASGRF1, a GTP exchange factor (GEF), is one of these genes. **Over-expression of Rasgrf1 is predicted to exacerbate neurofibromatosis while Rasgrf1 silencing will attenuate it.** Two novel strains of mice ideally suited to test this hypothesis that were developed in my lab are being used to evaluate the role or Rasgrf1 on the manifestations of neurofibromatosis type 1. One strain of mice over-express Rasgrf1, the other has diminished expression. These were crossed with a mouse model for NF1 and the effects of the altered level of RASGRF1 on tumorigenesis were monitored. The results indicate that over-expression of Rasgrf1 significantly hastens the time of tumor onset and increase the overall frequency of tumor incidence. In contrast, diminished expression modestly delays the timing of tumor development, but overall frequency of tumor development is not changed.

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Table of Contents

INTRODUCTION:

The goal of this research is to expand the knowledge of the genes that contribute to neurofibromatosis beyond the GAP1-related domain in NF1. It is hypothesized that the gene encoding RASGRF1, a GTP exchange factor (GEF), is one of these genes. **Over-expression of *Rasgrf1* is predicted to exacerbate neurofibromatosis while *Rasgrf1* silencing will attenuate it.** Two novel strains of mice ideally suited to test this hypothesis that were developed in my lab are being used to evaluate the role or *Rasgrf1* on the manisfestations of neurofibromatosis type 1.

BODY:

To test the influence or RASGRF1 on the manisfestations of neurofibromatosis type 1, we established crosses between a mouse model for NF1 and our mice that over- or under-express *Rasgrf1* (1) and Yoon et al. unpublished]. The NF1 model used is the so called "NP-cis" mice with lesions at *Nf1* and *p53* seven centimorgans apart on the same chromosome (2) Genotypic analysis of the progeny from this cross was done for *Nf1*, *p53* and the two separate alleles of *Rasgrf1*. A total of 123 animals were generated that included 75 with the original NP-cis genotype (NP), 25 mice with the NP-cis allele that also over-express *Rasgrf1* due to an activating mutation on the normally silent maternal allele (NP2) and 23 with the NP-cis allele that also under-express *Rasgrf1* due to an inactivating mutation on the single active paternal allele (NP3) and. The crosses were done in a manner that produced strain matched individuals so that the analysis of tumor incidence would not be confounded by strain background effects. The results indicate that over-expression of *Rasgrf1* in the NP2 animals significantly hastens the time of tumor onset and increases the overall frequency of tumor incidence. In contrast, diminished expression modestly delays the timing of tumor development, but overall frequency of tumor development is not changed (figure 1). These results demonstrate that *Rasgrf1* over-expression is a risk factor for tumorigenesis associated with the NP-cis mouse model of neurofibromatosis type 1.
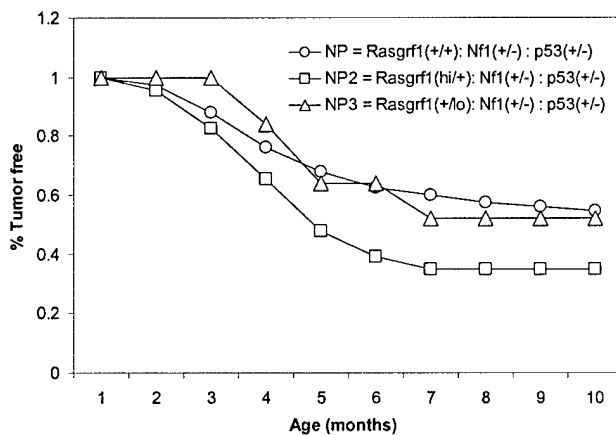

Figure 1. NP-cis mice (NP (2)) on the 129Sv background were bred with *Rasgrf1* mutant animals, also on the 129Sv background, with an inactivating lesion (Rasgrf1(lo/+) (1)) on the active paternal copy of this imprinted gene to produce NP3 animals, or the breeding was done with a transcription-activating mutation on the normally silent maternal allele (Rasgrf1(hi/+), Yoon et al. unpublished) to produce NP2 mice. All animals were monitored for 10 months after birth for signs of tumor formation. Mice were sacrificed shortly after tumor onset and tissue removed for later histological analysis.

The focus of ongoing work is to characterize histologically, the tumors that arose in the various genotypes of mice to determine if, beyond the quantitatively different kinetics and frequencies of tumor formation seen in the three genotypes of mice, there are also qualitative differences in the types of tumors that form.

4

KEY RESEARCH ACCOMPLISHMENTS:

- ☐ Development of the needed numbers and genotypes of mice needed to evaluate the role of RASGRF1 on tumor incidence in a neurofibromatosis type 1 model.
- ☐ Identification of over-expression of *Rasgrf1* as a contributing factor in tumor onset and frequency.
- ☐ Determination that loss of *Rasgrf1* expression produces no significant changes in tumor onset or frequency.
- ☐ Isolation of tumor tissues for histological analysis from NP-cis mice that are also manipulated for *Rasgrf1* expression.

REPORTABLE OUTCOMES:

Additional analysis of collected specimens is needed prior to reporting.

CONCLUSIONS:

This work demonstrates that in this animal model for neurofibromatosis type 1, over expression of *Rasgrf1* is a risk factor for faster tumor development and a higher frequency of tumor formation.

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